NATIONAL TOXICOLOGY PROGRAM
Technical Report Series
No. 403

# TOXICOLOGY AND CARCINOGENESIS

# STUDIES OF

# RESORCINOL

## (CAS NO. 108-46-3)

# IN F344/N RATS AND B6C3F$_1$ MICE

## (GAVAGE STUDIES)

U.S. DEPARTMENT OF HEALTH AND HUMAN SERVICES
Public Health Service
National Institutes of Health

## FOREWORD

The National Toxicology Program (NTP) is made up of four charter agencies of the U.S. Department of Health and Human Services (DHHS): the National Cancer Institute (NCI), National Institutes of Health; the National Institute of Environmental Health Sciences (NIEHS), National Institutes of Health; the National Center for Toxicological Research (NCTR), Food and Drug Administration; and the National Institute for Occupational Safety and Health (NIOSH), Centers for Disease Control. In July 1981, the Carcinogenesis Bioassay Testing Program, NCI, was transferred to the NIEHS. The NTP coordinates the relevant programs, staff, and resources from these Public Health Service agencies relating to basic and applied research and to biological assay development and validation.

The NTP develops, evaluates, and disseminates scientific information about potentially toxic and hazardous chemicals. This knowledge is used for protecting the health of the American people and for the primary prevention of disease.

The studies described in this Technical Report were performed under the direction of the NIEHS and were conducted in compliance with NTP laboratory health and safety requirements and must meet or exceed all applicable federal, state, and local health and safety regulations. Animal care and use were in accordance with the Public Health Service Policy on Humane Care and Use of Animals. The prechronic and chronic studies were conducted in compliance with Food and Drug Administration (FDA) Good Laboratory Practice Regulations, and all aspects of the chronic studies were subjected to retrospective quality assurance audits before being presented for public review.

These studies are designed and conducted to characterize and evaluate the toxicologic potential, including carcinogenic activity, of selected chemicals in laboratory animals (usually two species, rats and mice). Chemicals selected for NTP toxicology and carcinogenesis studies are chosen primarily on the bases of human exposure, level of production, and chemical structure. Selection *per se* is not an indicator of a chemical's carcinogenic potential.

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NTP TECHNICAL REPORT

ON THE

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NATIONAL TOXICOLOGY PROGRAM
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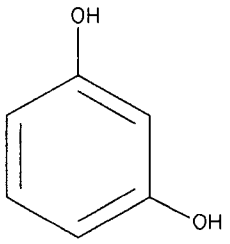
# CONTENTS

# ABSTRACT




**RESORCINOL**

CAS No. 108-46-3

Chemical Formula: $C_6H_6O_2$ Molecular Weight: 110.11

**Synonyms:** 1,3-benzenediol; *m*-dihydroxybenzene; resorcin

Resorcinol is used in the manufacture of adhesives and dyes and as an ingredient in pharmaceutical preparations for the topical treatment of skin conditions. Toxicity and carcinogenicity studies were conducted by administering resorcinol (>99% pure) in water by gavage to groups of F344/N rats and B6C3F$_1$ mice of each sex for 17 days, 13 weeks, and 2 years. Genetic toxicology studies were conducted in *Salmonella typhimurium*, Chinese hamster ovary cells, mouse lymphoma cells, and *Drosophila melanogaster*.

*17-Day Studies*: Groups of five rats of each sex were administered 0, 27.5, 55, 110, 225, or 450 mg/kg resorcinol and groups of five mice of each sex were administered 0, 37.5, 75, 100, 300, or 600 mg/kg resorcinol in deionized water by oral gavage. No rats died during the studies. All female and four male mice receiving 600 mg/kg and one male receiving 300 mg/kg died as a result of resorcinol administration. Final mean body weights of dosed rats and mice were similar to those of the control groups. No gross or microscopic lesions attributable to resorcinol administration were observed.

*13-Week Studies*: Groups of 10 rats of each sex were administered 0, 32, 65, 130, 260, or 520 mg/kg resorcinol and groups of 10 mice of each sex were administered 0, 28, 56, 112, 225, or 420 mg/kg resorcinol in deionized water by oral gavage. All female and eight male rats receiving 520 mg/kg and eight mice of each sex receiving 420 mg/kg resorcinol died of chemical-related toxicity during the studies. The final mean body weights of dosed rats and mice were similar to those of the control groups. No chemical-related gross or microscopic lesions were observed.

*2-Year Studies*: Doses were selected for the 2-year studies based on the decreased survival observed in the 13-week studies. Groups of 60 male rats and male and female mice were administered 0, 112, or 225 mg/kg resorcinol in deionized water by gavage, five days per week for up to 104 weeks. Groups of 60 female rats were initially administered the same doses as male rats, but by week 22 of the study 16 of the high-dose females had died. Consequently, the female rat study was restarted using doses of 0, 50, 100, or 150 mg/kg. After 15 months of exposure interim evaluations were performed on 10 animals from each group. No chemical-related changes in clinical pathology parameters or incidence of neoplasms or nonneoplastic lesions were found during the 15-month interim evaluations.

*Body Weights and Survival in the 2-Year Studies*: Mean body weights of high-dose male rats were 10% to 15% lower than those of the controls from

week 87 to study termination. Mean body weights of high-dose female rats were 11% to 14% lower than those of controls from week 95 to study termination. Mean body weights of other dosed rat groups were similar to those of controls. Survival of high-dose male and female rats was significantly lower than controls. Decreased survival in high-dose groups was attributed to chemical-related toxicity.

Mean body weights of high-dose female mice were 10% to 15% lower than those of controls from week 85 to study termination, whereas those of the remaining dosed mouse groups were similar to those of the controls. Survival of dosed mice was similar to that of controls. Clinical signs suggestive of a chemical-related effect on the central nervous system, including ataxia, recumbency, and tremors, were observed in rats and mice in the 2-year studies.

*Neoplasms and Nonneoplastic Lesions in the 2-Year Studies*: There were no treatment-related increased incidences of neoplasms or nonneoplastic lesions in rats or mice administered resorcinol for 2 years.

Mammary gland fibroadenomas occurred at significantly reduced incidences in all exposed groups of female rats (25/50, 14/50, 12/50, 9/50). The incidence of subcutaneous fibroma or sarcoma in high-dose male mice was significantly lower than for the controls (8/50, 6/50, 1/50).

*Genetic Toxicity*: Resorcinol was not mutagenic in *Salmonella typhimurium* strains TA98, TA100, TA1535, or TA1537 with or without exogenous metabolic activation (S9). Induction of trifluorothymidine resistance was observed in mouse L5178Y lymphoma cells treated with resorcinol in the absence of S9 activation; this test was not performed with S9. Resorcinol induced sister chromatid exchanges in Chinese hamster ovary cells with and without S9. Resorcinol was positive for induction of chromosomal aberrations in Chinese hamster ovary cells in the presence of S9; an equivocal response was obtained in this test in the absence of S9. No induction of sex-linked recessive lethal mutations was observed in the germ cells of male *Drosophila melanogaster* when resorcinol was administered in the feed, but an equivocal response was observed when the chemical was administered by injection.

*Conclusions*: Under the conditions of these 2-year gavage studies, there was *no evidence of carcinogenic activity** of resorcinol in male F344/N rats given 112 or 225 mg/kg or female F344/N rats given 50, 100, or 150 mg/kg. There was *no evidence of carcinogenic activity* of resorcinol in male or female B6C3$F_1$ mice given 112 or 225 mg/kg.

Clinical signs suggestive of a chemical-related effect on the central nervous system, including ataxia, recumbency, and tremors, were observed in rats and mice in the 2-year studies.

* Explanation of Levels of Evidence of Carcinogenic Activity is on page 8. A summary of the peer review comments and the public discussion of this Technical Report appear on page 10.

## Summary of the 2-Year Carcinogenicity and Genetic Toxicology Studies of Resorcinol

| | Male F344/N Rats | Female F344/N Rats | Male $B6C3F_1$ Mice | Female $B6C3F_1$ Mice |
|---|---|---|---|---|
| **Doses** | 0, 112, or 225 mg/kg in water by gavage 5 days a week | 0, 50, 100, or 150 mg/kg in water 5 days a week | 0, 112, or 225 mg/kg in water by gavage 5 days a week | 0, 112, or 225 mg/kg in water by gavage 5 days a week |
| **Body weights** | High-dose group lower than controls | High-dose group lower than controls | Dosed groups similar to controls | High-dose group lower than controls |
| **2-Year survival rates** | 28/50, 25/50, 9/50 | 34/50, 33/50, 28/50, 24/50 | 37/50, 43/50, 34/50 | 38/49, 33/49, 34/50 |
| **Nonneoplastic effects** | None | None | None | None |
| **Neoplastic effects** | None | None | None | None |
| **Level of evidence of carcinogenic activity** | No evidence | No evidence | No evidence | No evidence |
| **Genetic toxicology** | | | | |
| *Salmonella typhimurium* gene mutation: | Negative with and without S9 in strains TA98, TA100, TA1535, and TA1537 | | | |
| L5178Y mouse lymphoma gene mutation: | Positive without S9 | | | |
| Sister chromatid exchange | | | | |
| Chinese hamster ovary cells *in vitro*: | Positive with and without S9 | | | |
| Chromosomal aberrations | | | | |
| Chinese hamster ovary cells *in vitro*: | Positive with S9<br>Equivocal without S9 | | | |
| Sex-linked recessive lethal mutations | | | | |
| *Drosophila melanogaster* male germ cells: | Negative when administered in feed<br>Equivocal when administered by injection | | | |

# EXPLANATION OF LEVELS OF EVIDENCE OF CARCINOGENIC ACTIVITY

The National Toxicology Program describes the results of individual experiments on a chemical agent and notes the strength of the evidence for conclusions regarding each study. Negative results, in which the study animals do not have a greater incidence of neoplasia than control animals, do not necessarily mean that a chemical is not a carcinogen, inasmuch as the experiments are conducted under a limited set of conditions. Positive results demonstrate that a chemical is carcinogenic for laboratory animals under the conditions of the study and indicate that exposure to the chemical has the potential for hazard to humans. Other organizations, such as the International Agency for Research on Cancer, assign a strength of evidence for conclusions based on an examination of all available evidence including: animal studies such as those conducted by the NTP, epidemiologic studies, and estimates of exposure. Thus, the actual determination of risk to humans from chemicals found to be carcinogenic in laboratory animals requires a wider analysis that extends beyond the purview of these studies.

Five categories of evidence of carcinogenic activity are used in the Technical Report series to summarize the strength of the evidence observed in each experiment: two categories for positive results (**clear evidence** and **some evidence**); one category for uncertain findings (**equivocal evidence**); one category for no observable effects (**no evidence**); and one category for experiments that because of major flaws cannot be evaluated (**inadequate study**). These categories of interpretative conclusions were first adopted in June 1983 and then revised in March 1986 for use in the Technical Reports series to incorporate more specifically the concept of actual weight of evidence of carcinogenic activity. For each separate experiment (male rats, female rats, male mice, female mice), one of the following quintet is selected to describe the findings. These categories refer to the strength of the experimental evidence and not to potency or mechanism.

- **Clear evidence of carcinogenic activity** is demonstrated by studies that are interpreted as showing a dose-related (i) increase of malignant neoplasms, (ii) increase of a combination of malignant and benign neoplasms, or (iii) marked increase of benign neoplasms if there is an indication from this or other studies of the ability of such tumors to progress to malignancy.
- **Some evidence of carcinogenic activity** is demonstrated by studies that are interpreted as showing a chemical-related increased incidence of neoplasms (malignant, benign, or combined) in which the strength of the response is less than that required for clear evidence.
- **Equivocal evidence of carcinogenic activity** describes studies that are interpreted as showing a marginal increase of neoplasms that may be chemical related.
- **No evidence of carcinogenic activity** is demonstrated by studies that are interpreted as showing no chemical-related increases in malignant or benign neoplasms.
- **Inadequate study of carcinogenic activity** is demonstrated by studies that because of major qualitative or quantitative limitations cannot be interpreted as valid for showing either the presence or absence of carcinogenic activity.

When a conclusion statement is selected for a particular experiment, consideration must be given to key factors that would extend the actual boundary of an individual category of evidence. This should allow for incorporation of scientific experience and current understanding of long-term carcinogenesis studies in laboratory animals, especially for those evaluations that may be on the borderline between two adjacent levels. These considerations should include:

- adequacy of the experimental design and conduct;
- occurrence of common versus uncommon neoplasia;
- progression (or lack thereof) from benign to malignant neoplasia as well as from preneoplastic to neoplastic lesions;
- some benign neoplasms have the capacity to regress but others (of the same morphologic type) progress. At present, it is impossible to identify the difference. Therefore, where progression is known to be a possibility, the most prudent course is to assume that benign neoplasms of those types have the potential to become malignant;
- combining benign and malignant tumor incidences known or thought to represent stages of progression in the same organ or tissue;
- latency in tumor induction;
- multiplicity in site-specific neoplasia;
- metastases;
- supporting information from proliferative lesions (hyperplasia) in the same site of neoplasia or in other experiments (same lesion in another sex or species);
- presence or absence of dose relationships;
- statistical significance of the observed tumor increase;
- concurrent control tumor incidence as well as the historical control rate and variability for a specific neoplasm;
- survival-adjusted analyses and false positive or false negative concerns;
- structure-activity correlations; and
- in some cases, genetic toxicology.

# PEER REVIEW PANEL

The members of the Peer Review Panel who evaluated the draft Technical Report on the gavage studies of resorcinol on March 11, 1991, are listed below. Panel members serve as independent scientists, not as representatives of any institution, company, or governmental agency. In this capacity, Panel members have five major responsibilities:

- to ascertain that all relevant literature data have been adequately cited and interpreted,
- to determine if the design and conditions of the NTP studies were appropriate,
- to ensure that the Technical Report presents the experimental results and conclusions fully and clearly,
- to judge the significance of the experimental results by scientific criteria, and
- to assess the evaluation of the evidence of carcinogenic activity and other observed toxic responses.

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# SUMMARY OF PEER REVIEW COMMENTS

On March 11, 1991, the draft Technical Report on the toxicology and carcinogenesis studies of resorcinol received public review by the National Toxicology Program Board of Scientific Counselors' Technical Reports Review Subcommittee and associated Panel of Experts. The review meeting was held at the National Institute of Environmental Health Sciences, Research Triangle Park, NC.

Dr. M.P. Jokinen, NIEHS, introduced the toxicology and carcinogenesis studies of resorcinol by discussing the uses and rationale for study, describing the experimental design, reporting on survival and body weight effects, and commenting that there were no chemical-related nonneoplastic or neoplastic lesions in rats or mice. The proposed conclusions were *no evidence of carcinogenic activity* of resorcinol in rats or mice.

Dr. Klaassen, a principal reviewer, agreed with the proposed conclusions. He proposed that NTP measure blood levels of this chemical and others at various time points. Dr. R.D. Irwin, NIEHS, responded that NTP is now routinely incorporating the evaluation of blood levels as well as some basic pharmacokinetic parameters in 2-year studies and in many short-term toxicity studies.

Dr. Hayden, the second principal reviewer, agreed with the proposed conclusions. However, he questioned the adequacy of the study in male rats, noting the reduced survival in the 225 mg/kg dose group. Dr. J.K. Haseman, NIEHS, said that survival in this group was probably sufficient to detect a strong carcinogenic effect, and that survival in the 112 mg/kg dose group was unaffected, supporting the adequacy of the study for evaluating carcinogenicity. Dr. Hayden commented that he was struck by the apparent, and perhaps cumulative, neurotoxicity and suggested that a statement be added to the conclusions noting that clinical findings indicative of chemical-related toxicity to the central nervous system were observed. Dr. Jokinen responded that high-dose rats in the 2-year study seemed to have exaggerated clinical signs by the end of each 5-day dosing period, which might suggest effects on the central nervous system, although there were no morphologic lesions observed to support this. Dr. Hayden said this does not negate the possibility of interference with neurotransmitters. As to possible cumulative toxicity, Dr. Jokinen said chemical disposition studies indicated resorcinol was rapidly cleared from the blood and about 90% was excreted within 24 hours, primarily as a conjugate in the urine.

Further discussion ensued as to whether resorcinol could be considered to be a neurotoxin. Dr. Irwin said the observations were primarily empirical and he would have reservations about calling it a neurotoxin. Dr. Carlson urged caution in defining resorcinol as a neurotoxin without evidence that included a dose-response relationship. Mr. Beliczky reported that there is considerable exposure of workers in the rubber products industry where resorcinol has been used as part of an adhesive system.

Dr. Klaassen moved that the Technical Report on resorcinol be accepted with the revisions discussed and the conclusions as written for male and female rats and mice, *no evidence of carcinogenic activity.* Dr. Garman seconded the motion. Mr. Beliczky offered an amendment that the neurotoxic effects of resorcinol be addressed in the Conclusions. Dr. Hayden seconded the amendment. Dr. Haseman suggested that a statement addressing these concerns could be added to the Abstract, as well as the Conclusions: "Clinical signs suggestive of a chemical-related effect on the central nervous system, including ataxia, recumbency, and tremors, were observed in rats and mice in the 2-year studies." Dr. Hayden agreed. The amendment was accepted by seven yes votes to three no votes (Drs. Bailey, Goodman, and Klaassen). The original motion was then accepted unanimously with ten votes.